Application Note

An effective analytical procedure for gradual control of recombinant human insulin production

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THE MANUFACTURE OF protein and peptide pharmaceuticals is more complex than the isolation and purification of native or recombinant proteins from bacterial cells for in vitro use or diagnostics. The main difference is that only the biological activity of enzymes is standardized exactly without rigid requirements for protein purity and primary structure. For example, the addition of an affinity tag at the N- or C-terminus of a recombinant protein, in many cases, does not affect the biological activity of enzymes.[1] At the same time, biological activity, purity, primary structure, and several other parameters[2,3] of a protein or a peptide drug must satisfy *Pharmacopoeia* requirements. In the case of insulin, which, thus far, is the most effective pharmaceutical for the treatment of diabetes mellitus, the content of each specific impurity (i.e., proinsulin, insulin, polymers, and desamido-Asn^{a21}-insulin[2,3]) must also be determined.

In most instances, the developer of a new biotechnological process solves a number of typical problems. The most important include: reaching maximum conversion of intermediate products at each step of the procedure, determining an intermediate purity after each isolation and purification step, identifying contaminants in intermediates and final product, and confirming the primary structure and biological activity of the protein obtained. In other words, a high yield of pure end product can only be achieved by optimizing each technology step.

Even very effective and commonly used procedures, such as one-step conversion of insulin precursor to insulin with trypsin and carboxypeptidase B,[4–6] described by Kemmler,[7] should be performed with care. The authors' studies and published data[8,9] show that, depending on the precursor structure, or in the case of an unsuitable amount of proteases, a significant yield (up to 15%)[9] of an undesirable byproduct (i.e., des-Thr^{B30}-insulin) is obtained in the reaction. des-Thr^{B30}-insulin is one of the most difficult impurities to be removed from human insulin,[9] and its content (> than 2% in insulin preparation) exceeds a level of insulin-like contaminants specified by the *Pharmacopoeias*.[2,3]

Thus, careful control of each step of recombinant human insulin (rhI) production, as well as the manufacture of other recombinant proteins, is very important in the development of a proper and cost-effective technological process.

To evaluate a composition of complex protein and peptide samples at each technological step, the approach has to include separation techniques and methods of identification. Current separation techniques satisfy a number of requirements such as high selectivity, high sensitivity, high efficiency, high speed,[10] and minimal cost per analysis. At present, high-performance capillary electrophoresis (HPCE) and narrow-bore HPLC are the most powerful separation techniques that can be applied to resolve peptides and proteins with very similar structures.[11] Because of high reproducibility and precise quantitative estimation of separated compounds, these methods are used frequently in cross-validation studies during method validation[12] in analytical laboratories. Moreover, a combination of the two separation techniques based on different retention mechanisms (charge-to-mass ratio and hydrophobicity) provides unambiguous information about a sample's composition, which is essential for quality control of pharmaceuticals.[13] Other techniques, such as slab gel electrophoresis, are less suitable for these purposes because protein or peptide staining on gels does not provide precise quantitation.[14] For the identification of separated peptides, off-line matrix-assisted laser desorption ionization time-of-flight mass spectrometry[15] (MALDI-TOF-MS) was used. This technique allows the determination of exact molecular weights of peptides commonly used in proteomics for the identification of peptides and proteins.[16,17]

This application note illustrates the efficiency of the analytical scheme, which includes HPLC, HPCE, and MALDI-TOF-MS for the step-by-step control of rhI production.